

# Prevalence of Antibodies to Human Caliciviruses (HuCVs) in Kuwait Established by ELISA Using Baculovirus-Expressed Capsid Antigens Representing Two Genogroups of HuCVs

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Baculovirus recombinant-expressed antigens of Norwalk viruses (rNV) and a Mexico strain (rMX) of the Snow Mountain serogroup of human caliciviruses (HuCVs) were used in enzyme immunoassays to study the antibody prevalence among the Kuwaiti population and foreign workers employed in Kuwait. The antibody titers in 16 different age groups which ranged from neonates to centenarians were investigated by testing eight different dilutions of each serum (1:200–1:25,600). The results indicate that NV infection is widespread in Kuwait and affects all age groups. Ninety-eight percent of the 433 serum samples tested had antibodies to rNV. In the 50–79-year-old age group, the antibody levels to rNV were higher and significantly different from those in children 0–7 years old. In infants, the rNV antibodies did not diminish by 4 months of age and their titer steadily increased with age. When 414 of these sera samples were tested for antibodies to rMX, 96% positive serological responses were observed. Antibody titers to rMX were reduced in infants from 4 to 11 months; however, 95% of the samples were positive. These data indicate that children born in Kuwait are infected with Norwalk-like viruses at a very early age. Finally, antibodies to rNV and rMX were found in 98% of 151 and in 95% of 148 foreign workers, respectively. *J Med Virol* 51:115–118, 1997.

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was first identified in stool filtrates of affected people during an outbreak of gastroenteritis in Norwalk, Ohio, over 20 years ago [Kapikian et al., 1972]. Previous studies estimated that at least 42% of outbreaks of nonbacterial gastroenteritis in the United States are caused by Norwalk and Norwalk-like viruses [Kaplan et al., 1982], and these viruses are widely recognized agents of outbreaks of food-borne and waterborne viral gastroenteritis [CDC, 1990; Hedberg and Osterholm, 1993; Herwaldt et al., 1994].

All attempts to propagate the virus in cell culture have been unsuccessful, and consequently only a limited number of researchers have been able to develop diagnostic Norwalk assays using finite viral material from experimentally infected volunteers. The recent cloning and expression of NV-specific cDNA in insect cells infected with a baculovirus recombinant containing the viral capsid gene [Jiang et al., 1990, 1993] have made available high yields of immunologically reactive recombinant Norwalk virus-like particles (rNV VLPs). These VLPs are composed of a single capsid protein which spontaneously self assembles [Jiang et al., 1992]. A second antigenically distinct virus from Mexico (MX virus) that is Snow Mountain (SM)-like was cloned, expressed in a baculovirus expression system, and spontaneously folded into VLPs [Jiang et al., 1995a]. Similar to the rNV particles, the rMX VLPs were shown to be immunologically reactive by ELISA [Jiang et al., 1995a].

Seroepidemiological studies to determine the age prevalence of NV antibodies have been performed with samples collected in several countries including the United States [Blacklow et al., 1979; Greenberg et al., 1979], Japan [Numata et al., 1994], Thailand [Echever-

## INTRODUCTION

Norwalk virus (NV) is the prototype of the Caliciviridae, a family of morphologically similar viruses. NV

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ria et al., 1983], Panama [Ryder et al., 1985], Taiwan and the Philippines [Cukor et al., 1980], Bangladesh [Black et al., 1982], England [Gray et al., 1993; Parker et al., 1994], Finland [Lew et al., 1994b], Sweden [Hinkula et al., 1995], Mexico [Jiang et al., 1995b], Indonesia and Papua New Guinea [Numata et al., 1994]. Even remote ethnic groups such as the Bedouins in Saudi Arabia, Australian Aborigines [Parker et al., 1994], and Amerindians in Brazil [Gabbay et al., 1994] had positive serological reactivity to NV. Evidence for NV infection also has been found in South Africa [Taylor et al., 1993] and 10% of the cases of acute diarrhea among U.S. military personnel deployed to South America and West Africa were attributed to NV infection [Bourgeois et al., 1993]. Taken together, it appears that NV infections occur worldwide and may be important in widespread epidemic outbreaks of gastroenteritis.

Very little is known about the prevalence of antibodies to NV among civilians in the Middle East. Limited information confirming the presence of NV antibodies in Saudi Arabia and Israel was reported based on testing a small number of samples [Parker et al., 1994]. The presence of NV in the region was confirmed among U.S. military personnel participating in Desert Storm and Desert Shield operations [Hyams et al., 1993; Lew et al., 1994a]. The present study was designed to determine the prevalence and pattern of antibodies to rVLPs representing viruses in two different serogroups of human caliciviruses (HuCVs), NV and MX, among the civilian population living in Kuwait.

## MATERIALS AND METHODS

### Serum Samples

Two hundred and twenty two samples from adults and adolescents were obtained from outpatients without symptoms of gastroenteritis who were referred to the biochemistry laboratory at the Mubarak Hospital in Kuwait for different evaluations requiring blood samples. Of the adult sera, 139 were from foreign workers and 83 from Kuwaitis. More than 60% of the Kuwaiti population consists of foreign workers from more than 100 nationalities from all parts of the world. The serum samples from children were obtained from hospitalized patients. Twenty samples were from patients with gastroenteritis and paired sera were obtained in nine cases. Two hundred and eleven samples were obtained from patients hospitalized for other reasons. For the evaluation of antibody titers against rMX, 414 of the above-mentioned 433 sera were available for testing. In our sample analyses, the patients were divided into the following age groups: less than 4 months (m), 5–11 m, 12–23 m, 24–35 m, 3–4 years (y), 5–7 y, 8–10 y, 11–19 y, 22–29 y, 30–39 y, 40–49 y, 50–59 y, 60–69 y, 70–79 y, 80–89 y, and above 100 years.

### ELISAs

All sera were tested for the presence of antibodies to rNV and rMX in a recombinant VLP ELISA using the method described previously [Parker et al., 1993, 1994]. In brief, 96-well polyvinyl chloride, flat-bottomed Nunc-

Immuno Plate Maxi Sorp microplates were coated with 0.1 ml of rNV or rMX (1 µg/ml in 0.01 M PBS, pH 7.3) purified by density gradient centrifugation as described previously [Graham et al., 1994; Jiang et al., 1992, 1995b]. For detection of antibodies to rNV and rMX, we used horseradish peroxidase-conjugated affinity-purified goat-anti-human immunoglobulin (Ig)A, IgG, and IgM antibodies (Cappel Organon Teknika Corp., West Chester, NY) and the TMB (3,3',5,5'-tetramethylbenzidine) microwell peroxidase substrate system (Kirkegaard & Perry Laboratories Inc. Gaithersburg, MD). Pre and post-challenge sera were included in each plate as negative and positive controls [Graham et al., 1994]. Each serum sample was added to an antigen-coated well and to an uncoated but blocked well to control for nonspecific binding. Sera were tested at dilutions between 1:200 and 1:25,600, and an optical density (OD) of the coated well – OD uncoated well > two times the mean OD of two blanks and one negative serum control well (included in each plate) was considered to be a positive. All positives had an OD of >0.1. The mean titers and the standard error of the mean (SEM) for each age group were calculated against rNV and rMX.

## RESULTS AND DISCUSSION

Several viral agents cause acute gastroenteritis in humans. The etiological role of rotaviruses has been documented in Kuwait [Sethi et al., 1988], but very little is known about the prevalence of NV gastroenteritis in Kuwait and the Gulf region. During operation Desert Shield, NV was found to be the principal etiological agent of acute vomiting among troops stationed in Saudi Arabia, and serological evidence of NV infection was demonstrated among the military personnel [Hyams et al., 1993]. The nucleotide sequences of part of the polymerase region of two Desert Storm strains of NV were determined and found to be 73% identical to the corresponding region of the prototype NV [Lew et al., 1994a].

We used an ELISA in which rNV or rMX VLPs were used as antigen to determine the seroprevalence in Kuwait to these viruses. Previous studies have shown that rNV VLPs are efficient, specific, and more sensitive to detect serological responses to NV compared with the previously established techniques of immune electron microscopy (IEM) and blocking radioimmunoassay (RIA-BL) in which particle-positive fecal material from volunteers was used as the antigen [Green et al., 1993; Parker et al., 1993; Monroe et al. 1993].

The results from tests for rNV antibodies on 433 serum samples (222 from adults and 211 from children) are shown in Table I. The results from tests for rMX antibodies on 414 of the above-mentioned sera samples (215 from adults and 199 from children) are also shown in Table I. These data indicate that 98% of the individuals tested had antibodies to rNV and 96% had antibodies to rMX. All 222 sera from adults tested were positive for rNV antibodies and 95% of the sera from children 0–7 years old were positive for rNV antibodies. A high rate of antibodies against rMX was also found among adults (100%) and young children (0–7 years, 90%).

TABLE I. Prevalence, Titer, and Age Distribution of IgG Antibodies in Kuwait Against Two Different Serogroups of Norwalk Viruses, rNV and rMX

Mean titer <sup>a</sup>	No. positive/ no. tested		% Positive		Reciprocal of mean titer	
	rMX	rNV	rMX	rNV	rMX SEM <sup>b</sup>	rNV SEM
0–4 m	13/15	22/22	87	100	2,986 ± 397	4,120 ± 450
5–11 m	26/33	35/37	79	95	1,472 ± 535	4,100 ± 409
12–23 m	30/32	32/33	94	97	5,231 ± 999	4,100 ± 382
24–35 m	22/23	24/25	96	96	6,086 ± 1973	4,072 ± 849
3–4 y	29/30	28/31	97	90	8,520 ± 1472	4,240 ± 553
5–7 y	27/28	25/28	96	90	8,918 ± 1085	4,142 ± 468
8–10 y	34/35	34/35	97	97	5,833 ± 771	5,600 ± 784
11–19 y	22/23	23/23	96	100	6,656 ± 1047	5,820 ± 617
20–29 y	20/20	21/21	100	100	6,755 ± 1111	6,526 ± 975
30–39 y	52/52	54/54	100	100	8,476 ± 873	7,290 ± 675
40–49 y	43/43	43/43	100	100	10,741 ± 2246	7,530 ± 662
50–59 y	41/41	42/42	100	100	7,024 ± 2066	7,764 ± 660
60–69 y	21/21	20/20	100	100	6,495 ± 1460	8,876 ± 946
70–79 y	12/13	13/13	92	100	3,400 ± 1240	8,861 ± 1047
80–89 y	4/4	5/5	100	100	4,400 ± 2856	7,680 ± 2176
>100 y	1/1	1/1	100	100	6,400	6,400
Total <sup>c</sup>	397/414	422/433	96	98	6,077 ± 386	5,164 ± 197

<sup>a</sup>The mean titer for each age group was calculated by the summation of all values of the titers in a given group (positive and negative) and this figure was divided by the number of sera in each age group.

<sup>b</sup>Standard error of the mean.

<sup>c</sup>The total mean titer was computed by the summation of all values of titers for the study group and this figure was divided by the total number of sera tested.

To serologically confirm the presence of NV in Kuwait, four paired sera from children with gastroenteritis were tested. A fourfold rise of antibodies to rNV was found in the paired sera from one patient with gastroenteritis and equally high titers of 1:12,800 were found in the paired sera from the three additional patients.

The high prevalence of antibodies to rNV prompted titrating the level of antibodies in the different age groups. Sixteen age groups were evaluated for the presence of antibodies to rNV and rMX. These results (Table I) indicate that antibodies to rNV do not diminish by 4 months of age in children born in Kuwait and the level of antibodies rose with age. Consequently, the titer of antibodies to rNV in individuals from 50 to 79 years was significantly higher than in children 0–7 years (Tukey-HSD test,  $P = 0.05$ ). The titer of antibodies to rMX decreased among children (ages 0–11 months) and reached a peak among adults (age group 40–49 years). The decrease of antibodies to rMX, but not to rNV, might be explained by the persistence of NV-like viruses in Kuwait, or by SM-like strains similar to MX being introduced at a different time point, or both. Sequence analyses of viruses infecting children at various ages should help to distinguish between these possibilities.

These results indicate that children in Kuwait are infected with NV and MX-like viruses at a very early age. A high prevalence of serum antibody to rNV (85% at age 2 years) was found in Mexican children; antibody prevalence in the first week of life was 88%, declining to 53% in age group 7–9 months and increasing to 85% at 2 years of age [Jiang et al., 1995b]. The 90% prevalence of antibodies to rNV found in a 20-year-old group in Indonesia and the 100% prevalence in the 20–49-year-old group in Papua New Guinea [Numata et al.,

1994] suggest that infection with NV in developing countries may be common in childhood. In addition, a study in Finland has suggested that NV infection occurs very early in life [Lew et al., 1994b].

Our results demonstrating that a very high percentage of newborn babies and children in Kuwait have antibodies to NV contrast with results in children in the United States and Japan. The results in the United States were obtained before VLPs became available. The presence of antibodies to rNV was uncommon in childhood in these developed countries and only increased from school age to early adulthood [Blacklow et al., 1979; Greenberg et al., 1979; Numata et al., 1994]. The acquisition of antibodies to NV at an early age likely reflects a different pattern of exposure to NV in Kuwait. Higher seroprevalence rates early in life have been seen in sera from Ecuador, Bangladesh, the Philippines, and Brazil [Echeverria et al., 1983; Ryder et al., 1985; Cukor et al., 1980; Black et al., 1982; Gabbay et al., 1994].

The seroepidemiology of NV gastroenteritis in Kuwait is different from that in other parts of the world in that our results are the first to show 100% positivity to rNV among infants 0–4 months and among adults and adolescents older than 11 years old. In studies performed in children in Panama [Ryder et al., 1985], Taiwan and the Philippines [Cukor et al., 1980], the prevalence of NV infection ranged from 10% at 2 years of age in Taiwan to 98% at the age of 5 in Panama. In England, 60% of neonates possessed antibodies to NV [Gray et al., 1993; Parker et al., 1994]. Among adults, the seroprevalence in different regions of England was between 63 and 73% [Gray et al., 1993; Parker et al., 1994] while the prevalence among the Bedouins in Saudi Arabia

was 72% and among Australian Aborigines was 94% [Parker et al., 1994]. In our study in Kuwait, we also detected antibodies to rNV and rMX in foreign workers and their family members representing 20 different nationalities. The majority of these individuals were from developing countries where testing for Norwalk antibodies has not been performed (Afghanistan, Egypt, India, Iran, Iraq, Jordan, Lebanon, Pakistan, Syria, Greece, Somalia, Sri Lanka, Sudan, and Turkey). Antibodies to rNV and rMX were found in 98% of 151 samples tested and in 95% of 148 samples from foreign workers, respectively (data not shown).

This study has shown that the disease pattern of NV gastroenteritis in Kuwait differs from the pattern of disease in other parts of the world, and that children are heavily exposed to NV before passively acquired maternal antibodies have been cleared. The high antibody prevalence to HuCVs in serum samples from more than 200 children as reported here was unexpected. These new data concerning the seroprevalence of antibodies to two different HuCVs provide new insights about exposure to NV in Kuwait that will be of value to clinicians and may reduce the costly, unnecessary, and sometimes harmful administration of antibiotics to patients with diarrhea.

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